REMARKS

This amendment is being concurrently filed with an RCE.

Claims 103 has been amended. The application now includes claims 103 and 138-140.

The amendment to claim 103 overcomes the objection and provides antecedent basis for "said mammal".

Claim 103 has been amended to satisfy the requirements of 35 U.S.C. 112, first paragraph and second paragraph.

In particular, Claim 103 has been amended to recite specific protein sequences set forth in the application as filed. In particular, claim 103 references Seq. ID No. 8 (see page 15 of the sequence listing), Seq. ID No. 69 (see page 114 of the sequence listing), and Seq. ID No. 84 (see page 121 of the sequence listing). As such, claim 103 specifies the induction of an immune response against specific human hookworm antigens and utilizes Seq. ID Nos. to identify the polypeptide antigens. Table I (on page 15 et seq.) specifies each of the sequences now referenced in the claim. In addition, page 22, lines 17 et seq. specifically discusses using a larval stage antigen (e.g., *Na*-Asp-2) in combination with adult stage antigens (e.g., *Na*-Apr-1, and *Na*-CP-2), and page 22, line 30 specifies the particular combinations now being claimed. Pages 21 and 23 specifically discuss vaccination of a mammal or eliciting an immune response in the mammal, and page 22 references use of adjuvants.

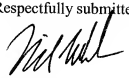
The Examples section beginning on page 26 provide considerable evidence demonstrating the utility of the invention. Since filing of the application, development of a vaccine including *Na*-Asp-2 has proceeded to clinical trials in humans, and additional research and testing has shown favorable results with a combination of *Na*-Asp-2 used in combination with adult stage antigens. Attached hereto is a summary of the testing which has proceeded based on the patent filing (test results being referenced in refereed journals). These results demonstrate vaccination with one of the antigens specified in the claims protects against hookworm infections and that bivalent vaccines will provide complementary modes of action.

In view of the above, claims 103 (as amended) and 138-140 satisfy the requirements of 35 U.S.C. 112, first paragraph and second paragraph, and should now be in condition for allowance. Reconsideration and allowance of the claims at an early date is requested.

Should the Examiner find the application to be other than in condition for allowance, the Examiner is requested to contact the undersigned at 703-787-9400 (fax: 703-787-7557; email: mike@wcc-ip.com) to discuss any other changes deemed necessary in a telephonic or personal interview.

If an extension of time is required for this response to be considered as being timely filed, a conditional petition is hereby made for such extension of time. Please charge any deficiencies in fees and credit any overpayment of fees to Attorney's Deposit Account No. 50-2041.

Respectfully submitted,



Michael E. Whitham
Reg. No. 32,635

Whitham, Curtis, Christofferson & Cook, P.C.
11491 Sunset Hills Road, Suite 340
Reston, VA 20190
703-787-9400 (Telephone)
703-787-7557 (Facsimile)

Vaccination with recombinant *Na*-ASP-2 protects against *Necator americanus* hookworm infection

Recombinant *Na*-ASP-2 was cloned from a Necator americanus L3 cDNA library (prepared from a Chinese strain of *N. americanus*) and expressed in the yeast, Pichia pastoris (Goud et al, 2005). In yeast, the recombinant *Na*-ASP-2 protein was expressed at high yield, and purified by two ionic-exchange chromatography steps followed by desalting on Sephadex G-25. The purified recombinant protein had a molecular mass (confirmed by mass spectrometry) of 21.3 kDa, with an N-terminal 6 amino acid vector tag to facilitate expression, and a single O-linked mannose (Goud et al, 2005). The crystal structure of *Na*-ASP-2 was also solved (Asojo et al, 2005).

Evidence that Na-ASP-2 is an effective hookworm vaccine is based on three lines of evidence:

1. The vaccine is a protective antigen in hamsters challenged with *Necator americanus*. For these studies, golden hamsters (*Mesocricetus auratus*) are vaccinated with with either 25 ug or 50 ug of purified recombinant protein mixed with either allhydrogel or the GlaxoSmithKline adjuvant AS03. A total of three immunizations were performed. The vaccine was administered subcutaneously in three injections at two week intervals. One week after the final immunization the hamsters are challenged with 150 infective third-stage larvae administered through the skin of the central abdomen. Twenty-five to 28 days post challenged, all hamsters of the vaccinated or control groups were sacrificed and the adult hookworms in the small intestine were collected and counted. The mean worm burden in each group was calculated; the differences of recovered worm number between the vaccinated group and control group were analyzed by using the Student t-test. Shown in Table 1 are the results of three different vaccine trials with recombinant *Na*-ASP-2. Vaccination of recombinant *Na*-ASP-2 formulated with AS03 elicited 40% worm burden reduction with statistical significance compared to the adjuvant control group. In the second trial, alhydrogel-formulated *Na*-ASP-2 provided a 46% worm burden reduction, however the protection was not statistically significant because of a large standard deviation in the vaccine and control groups. Therefore, a third trial was followed with alhydrogel-formulated *Na*-ASP-2 and a 30% worm burden reduction was achieved with statistical significance. Combining the results of the three trials together for a total of 64 hamsters in both the experimental and control groups, the mean worm burden reduction rate resulting from *Na*-ASP-2 vaccination was 37% with overall statistical significance compared with the adjuvant group.

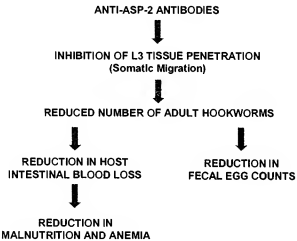
Table 1. Protective immunity elicited by immunizing recombinant *Na*-ASP-2 in hamsters challenged with *Necator americanus* third stage infective larvae

Trial	Vaccine Antigen	Adjuvant	Mean worm burden CONTROL	Mean worm burden VACCINATED	Worm burden REDUCTION	P Valuen
1	*Na*-ASP-2 25 ug	AS03	19.4 ± 14.6	11.7 + 9.2	40%	<0.05
2	*Na*-ASP-2 25 ug	Alhydrogel	16.9 ± 14.7	9.1 ± 10.1	46%	>0.05
3	*Na*-ASP-2 50 ug	Alhydrogel	37.7 ± 13.6	26.4 ± 17.2	30%	<0.05
TOTAL	*Na*-ASP-2		24.7 ± 16.6	15.5 ± 14.3	37.2	<0.01

2. A second important line of evidence that *Na*-ASP-2 is an effective vaccine is based on studies in rats. In Sprague Dawley rats doses of 50-150 ug of *Na*-ASP-2 formulated with alhydrogel induces a strong antibody response comprised of high specific IgG1, IgG2a, and IgM titers (Fujiwara et al, 2005). High IgG antibody titers were maintained for up to three months post vaccination, and were boosted by an additional vaccine dose. Specific cell proliferation and a Th2 cytokine profile were also observed in peripheral blood of the immunized rats. The rat-anti-*Na*-ASP-2 antibody recognizes native Na-ASP-2 on western blots and in vitro has the ability to inhibit *N. americanus* hookworm larvae from migrating through skin (Goud et al, 2005). The anti-*Na*-ASP-2 antibody inhibited migration 90 $\pm$ 7% in three different experiments compared with 17.7 $\pm$ 7% inhibition by serum from rats injected with alhydrogel alone (P = 0.019) (Goud et al, 2005).

   Thus, preclinical studies suggest that ASP-2 vaccines operate by eliciting host antibodies, which inhibit larval migration and reduce the number of L3 that develop into adult hookworms (Fig. 1)

Fig. 1. Proposed mechanism by which ASP-2 vaccination reduces worm burdens & blood loss



3. Finally, in an unpublished Phase 1 study in humans (manuscript in preparation), an Na-ASP-2 Hookworm Vaccine comprised of 10 ug, 50 ug, or 100 ug of recombinant protein formulated with alhydrogel elicited high titer IgG antibody. The human anti-Na-ASP-2 antiserum exhibited the ability to recognize native Na-ASP-2 in larval extracts as evidenced by immunoprecipitation followed by western blotting (IP-Westerns) (data not shown).

References for Na-ASP-2 Protection Studies

Asojo OA, Goud G, Dhar K, Loukas A, Zhan B, Deumic V, Liu S, Borgstahl G, Hotez P. 2005b. Novel X-ray structure of *Na*-ASP-2, a PR-1 protein from the nematode parasite *Necator americanus* and a vaccine antigen for human hookworm infection. *Journal of Molecular Biology* 346: 801-14.

Fujiwara R, Bethony J, Bueno L, Wang Y, Ahn S, Samuel A, Bottazzi A, Hotez PJ, Mendez S. 2005. Immunogenicity of the hookworm *Na*-ASP-2 vaccine candidate. *Human Vaccines* 2005; 1: 123-8.

Goud GN, Bottazzi ME, Zhan B, Mendez S, Deumic V, Pleiskatt J, Liu S, Wang Y, Bueno L, Fujiwara R, Samuel A, Ahn SY, Solanki M, Asojo O, Wen J, Saul A, Bethony JM, Loukas A, Roy M, Hotez PJ. 2005. Expression of the Necator americanus hookworm larval antigen *Na*-ASP-2 in *Pichia pastoris* and purification of the recombinant protein for use in human clinical trials. *Vaccine* 2005; 4754-64.

Vaccination with dog hookworm ASP-2, either *Ac*-ASP-2 or *Ay*-ASP-2 from Ancylostoma caninum or Ancylostoma ceylanicum, respectively also protects against challenge hookworm infections.

Recombinant ASP-2 molecules cloned from *A. caninum* (*Ac*-ASP-2), *A. ceylanicum* (*Ay*-ASP-2), were also tested as vaccines.

In dogs, recombinant *Ac*-ASP-2 formulated with the GSK adjuvant AS03 was a protective against *A. caninum* challenge infections as evidenced by a 26% reduction in worm burden and a 69% reduction in fecal egg counts (Bethony et al, 2005) relative to controls. Additional preclinical studies have shown that the protection afforded by immunizing dogs with irradiated third-stage *A. caninum* hookworm larvae is mediated by anti-*Ac*-ASP-2 antibodies (Bethony et al, 2005; Fujiwara et al, 2006).

In hamsters recombinant *Ay*-ASP-2 formulated with Quil A was also a protective antigen as evidenced by a 32% reduction in worm burden and a 55% reduction in host blood loss following challenge with *A. ceylanicum* hookworm larvae (Goud et al, 2004; Mendez et al, 2005).

References for Ac-ASP-2 and Ay-ASP-2 Protection studies

Bethony JM, Loukas A, Smout MJ, Mendez S, Wang Y, Bottazzi ME, Zhan B, Williamson AL, Lustigman S, Correa-Oliveira R, Xiao SH, Hotez PJ. 2005. Antibodies against a secreted protein from hookworm larvae reduce the intensity of infection in humans and vaccinated laboratory animals. *FASEB Journal* 19: 1743-5.

Fujiwara RT, Loukas A, Mendez S, Williamson AL, Bueno LL, Wang Y, Samuel A, Zhan B, Bottazzi ME, Hotez PJ, Bethony JM. 2006. Vaccination with irradiated *Ancylostoma caninum* third stage larvae induces a Th-2-like response in dogs. *Vaccine* 24: 501-9.

Goud GN, Zhan B, Ghosh K, Loukas A, Hawdon J, Dobardzic A, Deumic V, Liu S, Dobardzic R, Zook RC, Qun J, Liu YY, Hoffman L, Chung-Debose D, Patel R, Mendez S, Hotez PJ. 2004. Cloning, yeast expression, isolation and vaccine testing of recombinant Ancylostoma secreted protein 1 (ASP-1) and ASP-2 from *Ancylostoma ceylanicum*. *Journal of Infectious Diseases* 189: 919-29.

Mendez S, Zhan B, Goud G, Ghosh K, Dobardzic A, Wu WH, Liu S, Deumic V, Dobardzic R, Liu YY, Bethony J, Hotez PJ. 2005b. Effect of combining the larval antigens Ancylostoma secreted protein 2 (ASP-2) and metalloprotease 1 (MTP-1) in protecting hamsters against hookworm infection and disease caused by *Ancylostoma ceylanicum*. *Vaccine* 23: 3123-30.

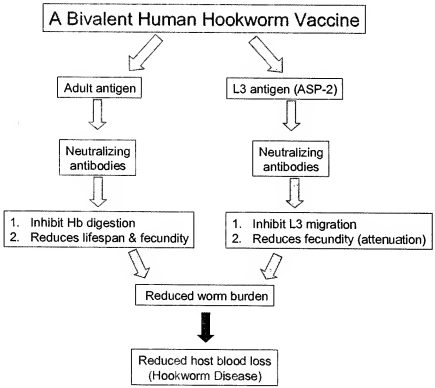
Rationale for development of a bivalent Human Hookworm Vaccine

Based on the preclinical studies outlined below, it is unlikely that vaccination with recombinant *Na*-ASP-2 formulated with an alhydrogel, and possibly other platform adjuvants such as CpGs will not prevent 100% of the infective hookworm larvae from migrating from the skin and entering the lungs and intestine. Therefore it is expected that a second antigen will be added to *Na*-ASP-2 in order to create a bivalent vaccine. The second antigen will be directed at the adult intestinal form of hookworms. Such an approach is analogous to the strategy of developing either bivalent or multivalent vaccines to produce "multiple layers" of immunity against malaria

(Shi et al, 1999). Three different "second antigens" are under development and evaluation. These include Na-APR-1 (Loukas et al, 2005), Na-GST-1 (Zhan et al, 2005), and Na-CP-2 (Loukas et al, 2004). Each of these vaccine antigens has been shown to either further reduce hookworm blood loss, egg fecundity, or hookworm burden (Loukas et al, 2004; 2005; Zhan et al, 2005).

Therefore the final Human Hookworm Vaccine will consist of *Na*-ASP-2 combined with either *Na*-APR-1, Na-GST-1, or Na-CP-2, together with one or more adjuvants, most likely Alhydrogel® with and additional immunostimulant such as CpGs. An example of how this bivalent strategy will work is shown in Fig. 2. It is expected that vaccination with the *Na*-ASP-2 L3 antigen will elicit antibodies that will inhibit larval migration and ultimately reduce the number of adult hookworms in the small intestine, whereas vaccination with second antigen will elicit antibodies, which will be ingested by the adult hookworms, and subsequently interfere with blood feeding and reduce blood loss at the site of parasite attachment. Therefore the two antigens are expected to complement each other's mode of action (Loukas et al, 2006).

Fig. 2. Proposed mode of action of the Human Hookworm vaccine



References for a bivalent human hookworm vaccine

Hotez PJ, Bethony J, Bottazzi ME, Brooker S, Diemert D, Loukas A. 2006. New technologies for the control of human hookworm infection. *Trends in Parasitology* 22: 327-31.

Loukas A, Bethony JM, Williamson AL, Goud GN, Mendez S, Zhan B, Hawdon JM, Bottazzi ME, Brindley PJ, Hotez PJ. 2004. Vaccination of dogs with recombinant cysteine protease from the intestine of canine hookworms diminishes fecundity and growth of worms. *Journal of Infectious Diseases* 189: 1952-61.

Loukas A, Bethony JM, Mendez S, Fujiwara RT, Goud GN, Ranjit N, Zhan B, Jones B, Bottazzi ME, Hotez PJ. 2005. Vaccination with recombinant aspartic hemoglobinase reduces parasite load and blood loss after hookworm infection. *PLoS Medicine 2: e295.*

Loukas A, Bethony J, Brooker S, Hotez P. 2006. Hookworm vaccines – past, present and future. *Lancet Infectious Diseases* 6: 733-41.

Shi YP, Hasnain SE, Scci JB, Holloway BP, Fujioka H, Kumar N, Wohlhueter R, Hoffman SL, Collins WE, Lal AA. 1999. Immunogenicity and in vitro protective efficacy of a recombinant multistage Plasmodium falciparum candidate vaccine. Proc Natl Acad Sci USA 96: 1167-9.

Zhan B, Liu S, Perally S, Fujiwara R, Brophy P, Liu YY, Feng JJ, Williamson A, Wang Y, Bueno LL, Mendez S, Goud G, Bethony JM, Hawdon JM, Loukas A, Jones K, Hotez PJ. 2005. Biochemical characterization and vaccine potential of a heme binding glutathione S transferase (GST) from the adult hookworm *Ancylostoma caninum Infection and Immunity* 73: 6903-11.